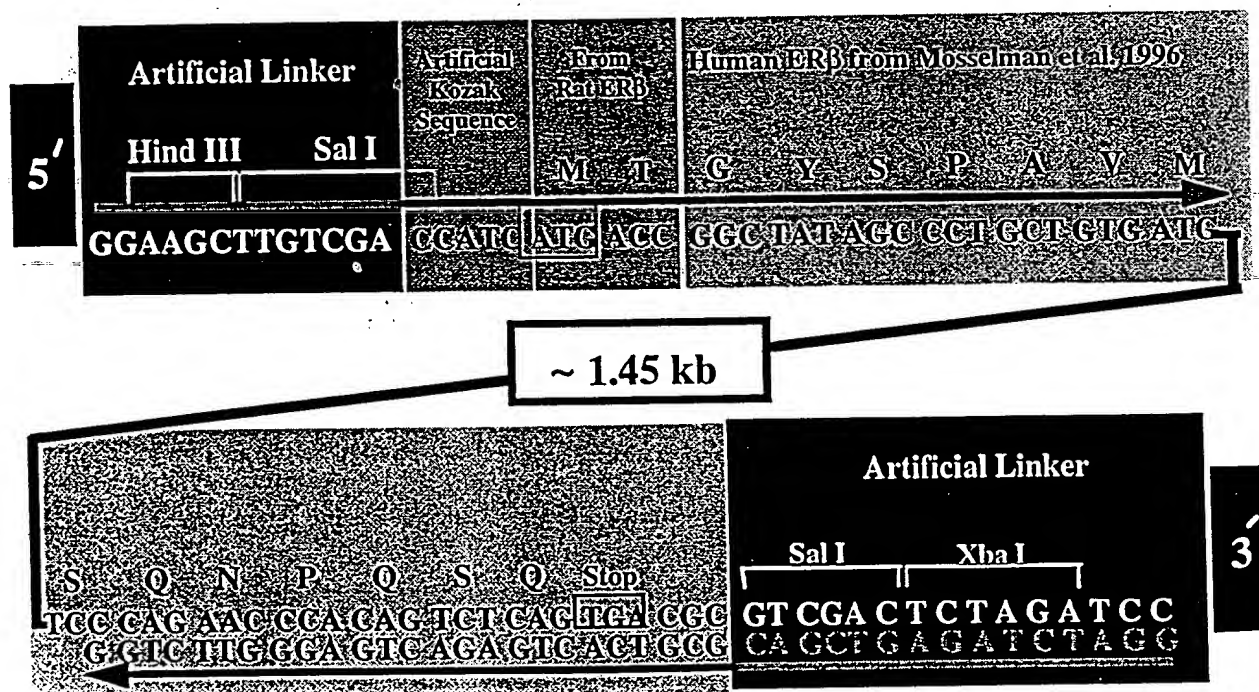


FIGURE 1

PCR Cloning of the Human ER β

Human testis RNA was reverse transcribed using Oligo dT. The resulted transcript was used for PCR with Oligonucleotides (red arrows) designed as follows:



The PCR product was cloned in the Hind III and Xba I sites of the Eukaryotic expression vector pcDNA III.

FIGURE 2

Human ER β cDNA

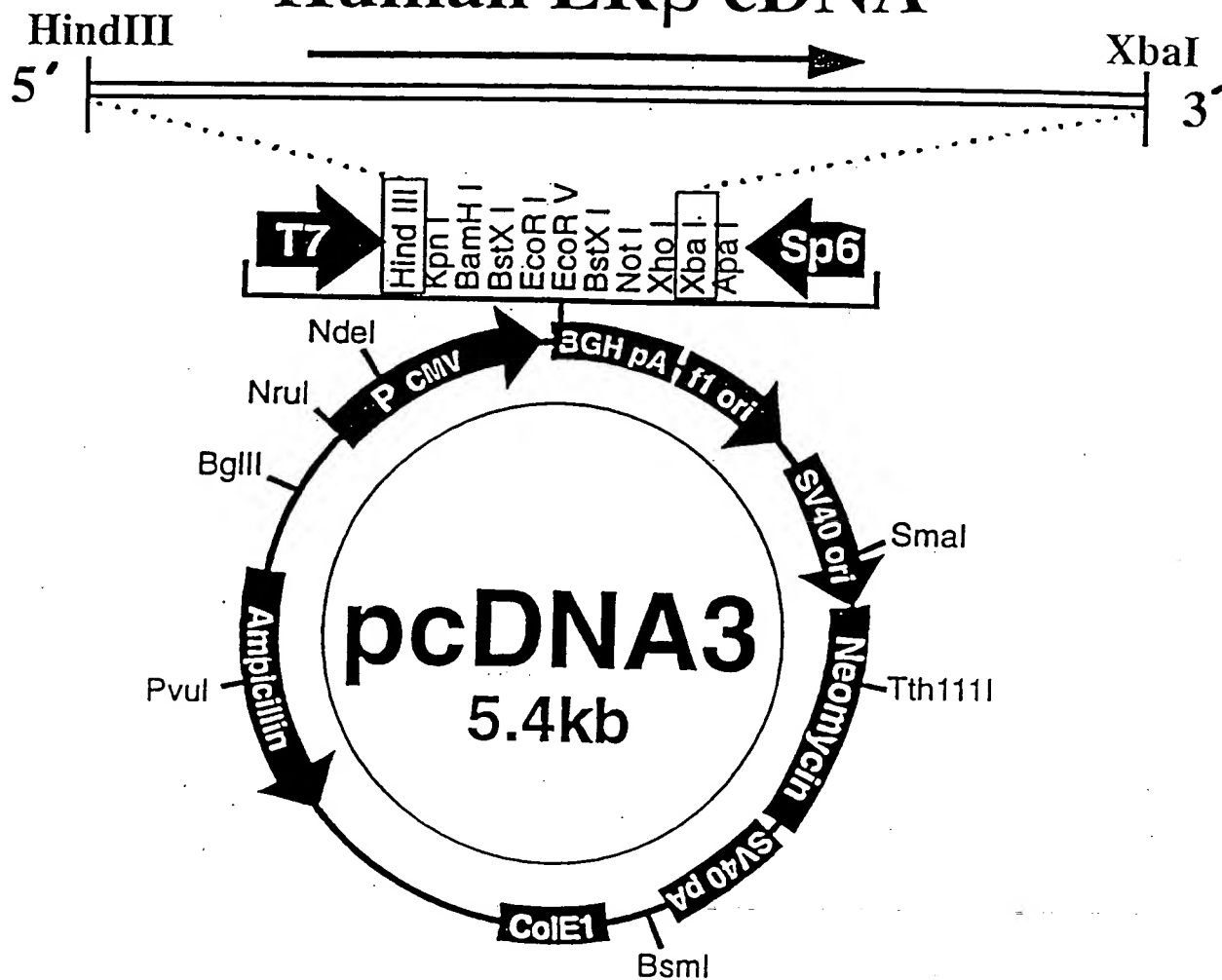


Fig 2

265080-59E9680

CAGOCATTATACTTGGOCAOGAATCTTTGAGAACATTATAATGACCTTTGTGCOCTCTTCT 60
TGCAAGGTGTTTTCTCAGCTGCTATCTCAAGACATGGATATAAAAACTCAOCCATCTAGC 120
CTTAATTCTOCTTOCTOCTACAACAGTCAATCCATCTTACCOCTGGAGCAOGGCTOC 180
ATATACATAOCTTOCTOCTATGTAGACAGCAOCCATGAATATCCAGCAATGACATTCTAT 240
AGOOCTGCTGTGATGAATTACAGCATTOCCAGCAATGTCACTAACTTGAAGGTGGGOCT 300
GGTOGGCAGAACACAAGOOCAAATGTGTTGTGGCAACAOCTGGGCAOCTTTCTOCTTTA 360
GTGGTOCATOGCCAGTTATCAGATCTGTATGOGGAACCTCAAAAGAGTOOCTGGTGTGAA 420
GCAAGATOGCTAGAACACAACCTTAOCTGTAAACAGAGAGACACTGAAAAGGAAGGTTAGT 480
GGGAACCGTTGOGOCAGOOCTGTTACTGGTOCAGGTTCAAAGAGGGATGCTCACTTCTGC 540
GCTGTCTGCAGOGATTACGCATCGGGATATCACTATGGAGTCTGGTOGTGTGAAGGATGT 600
AAGGCOCTTTTTTAAAAGAAGCATTCAAGGACATAATGATTATATTTGTCCAGCTACAAAT 660
CAGTGTACAATOGATAAAAAOOGGOGCAAGAGCTGOCAGGOCTGOOGACTTOGGAAGTGT 720
TAOGAAGTGGGAATGGTGAAGTGTGGCTOOOGGAGAGAGAGATGTGGGTACOGCOCTTGTG 780
OGGAGACAGAGAAGTGOOGAOGAGCAGCTGCACTGTGGOOGCAAGGOCAAGAGAAGTGGC 840
GGOCAOGOGOOOOGAGTGOGGGAGCTGCTGCTGGAOOGOOCTGAGOOOOGAGCAGCTAGTG 900
CTCAOCTOCTGGAGGCTGAGOOOGOOOCATGTGCTGATCAGOOOGOOOCAGTGOGOOCTTC 960
AOCGAGGOCTOCATGATGATGTCCCTGAOCCAAGTTGGOOGACAAGGAGTTGGTACACATG 1020
ATCAGCTGGGGOCAAGAAGATTCCOOGGCTTTGTGGAGCTCAGCOCTGTTGACCAAGTGOGG 1080
CTCTTGAGAGCTGTTGGATGGAGGTGTTAATGATGGGGCTGATGTGGOGCTCAATTGAC 1140
CACOOOGGCAAGCTCATCTTTGCTCCAGATCTTGTCTGGACAGGGATGAGGGGAAATGC 1200
GTAGAAGGAATTCTGGAAATCTTTGACATGCTOCTGGCAACTACTTCAAGGTTTOGAGAG 1260
TTAAAACTCCAACACAAAGAATATCTCTGTGTCAAGGOCATGATCCTGCTCAATTCCAGT 1320
ATGTACCOCTCTGGTCACAGOGAOCOCAGGATGCTGACAGCAGCOOGAAGCTGGCTCACTTG 1380
CTGAOOGOOGTGAOOGATGCTTTGGTTTGGGTGATTGCOAAGAGOGGCATCTOCTOOCAG 1440
CAGCAATCCATGOGCOCTGGCTAACCTOCTGATGCTOCTGTCCOAGTCAGGCATGOGAGT 1500
AACAAGGGCATGGAACATCTGCTCAACATGAAGTGCAAAAATGTGGTOCCAGTGTATGAC 1560
CTGCTGCTGGAGATGCTGAATGOOCAOGTGTTOGOGGGTGCAAGTOCTOCATCAOGGGG 1620
TOOGAGTGCAGOOOGGCAGAGGACAGTAAAAGCAAAGAGGGCTOCCAGAACCCACAGTCT 1680
CAGTGA 1686

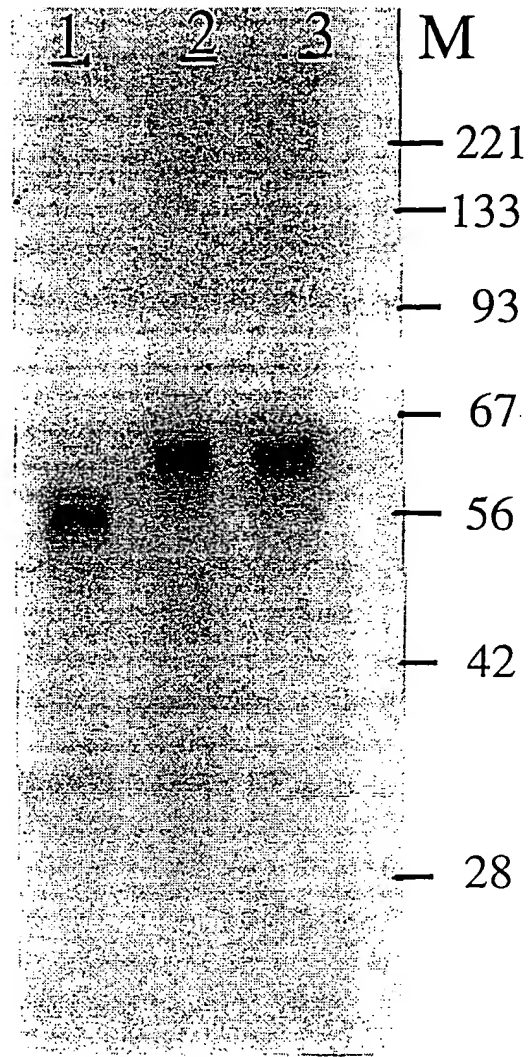
763

MDIKNSPSSL	NSPSSYNCSO	SILPLEHGSI	YIPSSYVDSH	HEYPAMIFYS	50
PAVMNYSIPS	NVINLEGGPG	RQTTSPNVLW	PTFGHLSPLV	VHRQLSHLYA	100
EPQKSPWCEA	RSLEHTLPVN	RETLKRKVSG	NRCASPVITGP	GSKRDAHFCA	150
VCSDYASGYH	YGVWSCEGCK	AFFKRSIQGH	NDYICPATNQ	CTIDKNRRKS	200
CQACRLRKCY	EVGVMKCGSR	RERCGYRLVR	RQRSADEQLH	CAGKAKRSGG	250
HAPRVRELLL	DALSPEQLVL	TLLEAEPPHV	LISRPSAPFT	EASMMMLTK	300
LADKELVHMI	SWAKKIPGFV	ELSLFDQVRL	LESCWMEVLM	MGLMWRSIDH	350
PGKLIFAPDL	VLDRDEGKCV	EGILEIFDML	LATTSRFREL	KLQHKEYLCV	400
KAMILLNSSM	YPLVTATQDA	DSSRKLAHLL	NAVTDALVWV	IAKSGISSQQ	450
QSMRLANLLM	LLSHVRHASN	KGMEHLLNMK	CKNVVPVYDL	LLEMLNAHVL	500
RGCKSSITGS	ECSPAEDSKS	KEGSONPQSQ	.	531	

Fig 4

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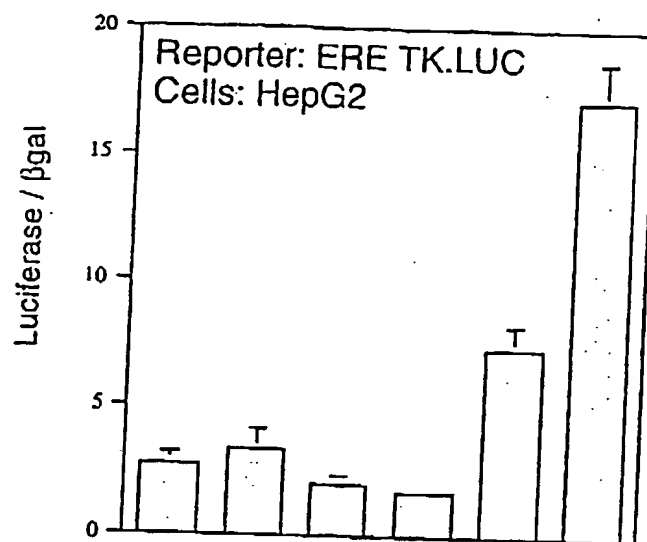
Fig 5



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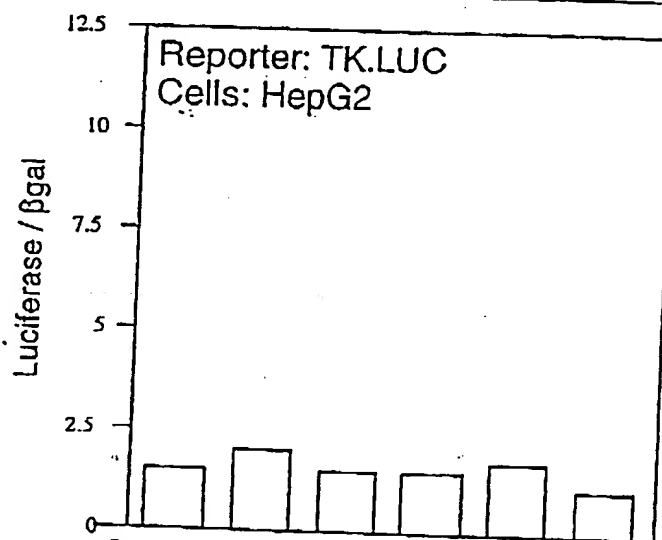
F766

A



17-β Estradiol (10^{-6})	-	+	-	-	+	+
hum ERβ _T	-	-	+	-	+	-
hum ERβ _L	-	-	-	+	-	+

B



17-β Estradiol (10^{-6})	-	+	-	-	+	+
hum ERβ _T	-	-	+	-	+	-
hum ERβ _L	-	-	-	+	-	+

Transactivation of ERE reporter By ERβ_L and ERβ_T In HepG2 cells. Luciferase reporter constructs (0.5 ug) containing either [A] the estrogen receptor DNA response element upstream of the TK basal promoter (ERE TK.LUC) or [B] the TK basal promoter alone (TK.LUC) were transiently transfected into HepG2 cells by the calcium phosphate coprecipitation method. Each construct was cotransfected with the ER expression vector (0.25 ug) indicated and the RSV-β-galactosidase plasmid (0.5 ug) to correct for variation in DNA uptake. Luciferase activity was normalized to β-galactosidase enzymatic activity.

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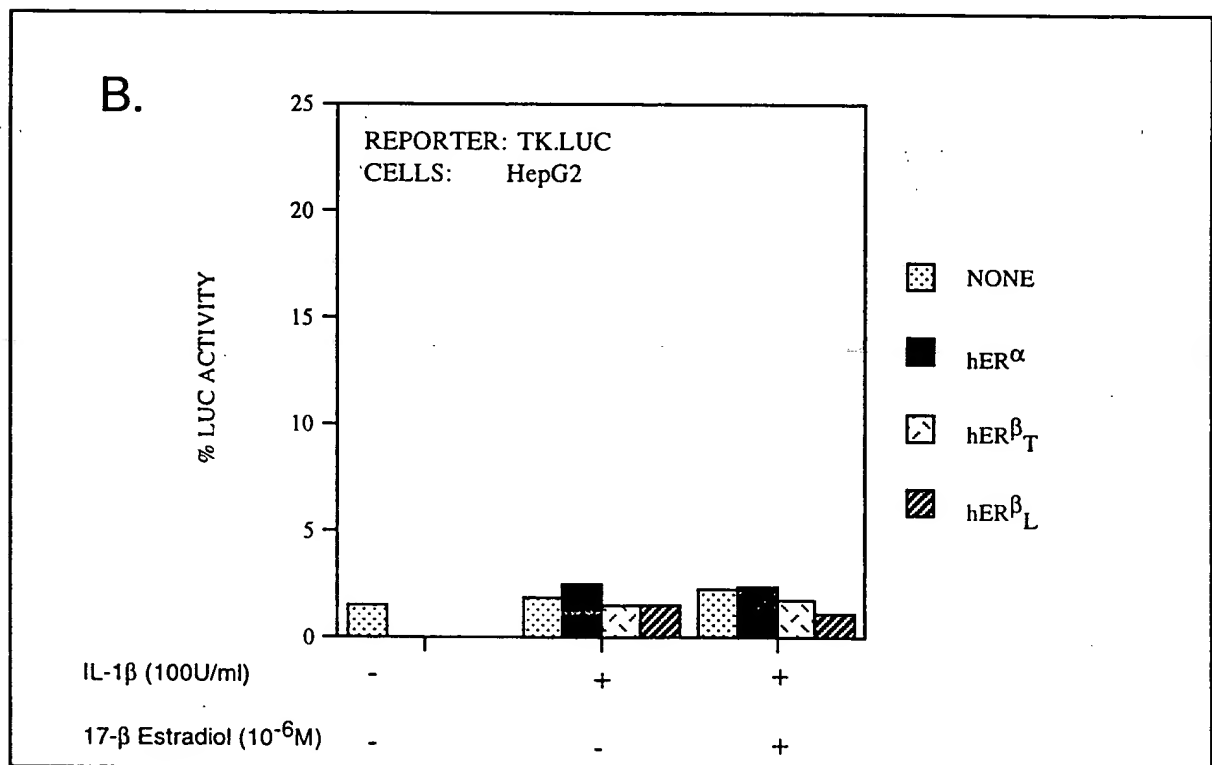
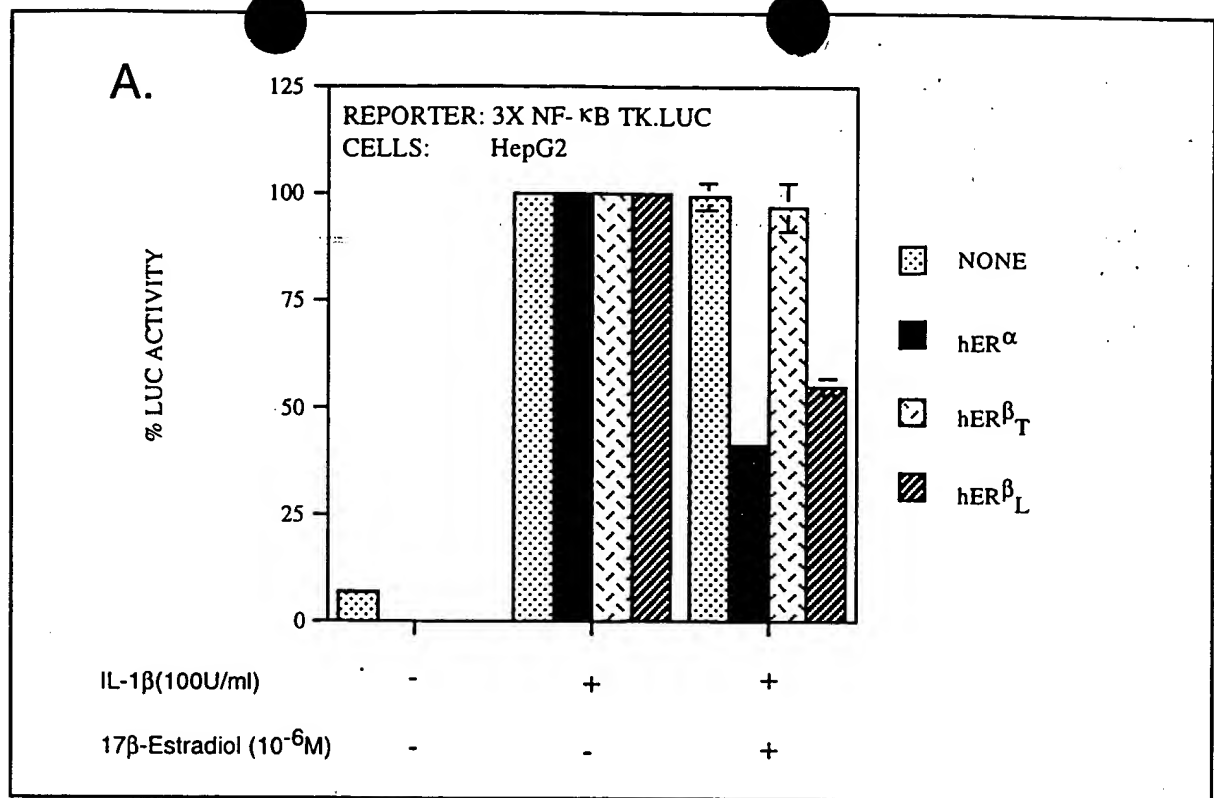
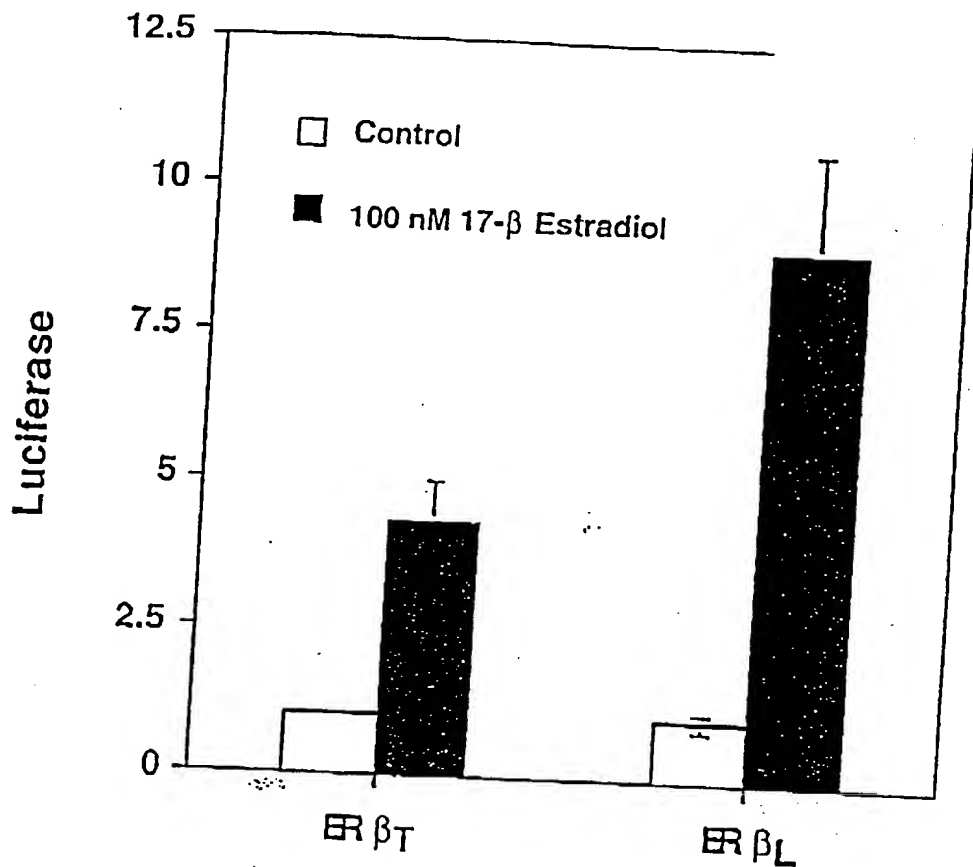


Fig 7

Luciferase reporter constructs (0.5 μ g) containing either [A] 3 copies of an NF κ B binding site upstream of the TK basal promoter (3X-NF κ B TK.LUC) or [B] the TK basal promoter construct alone (TK.LUC) were transiently cotransfected into HepG2 cells by the calcium phosphate coprecipitation method. Each construct was cotransfected with the ER expression vector indicated and the plasmid RSV- β -galactosidase (0.5 μ g) to correct for variation in DNA uptake. Percent luciferase activity values represent Luc: β -galactosidase enzymatic activity ratios relative to a value of 100% designated for the IL-1 β treated samples and are presented as mean \pm S.E..

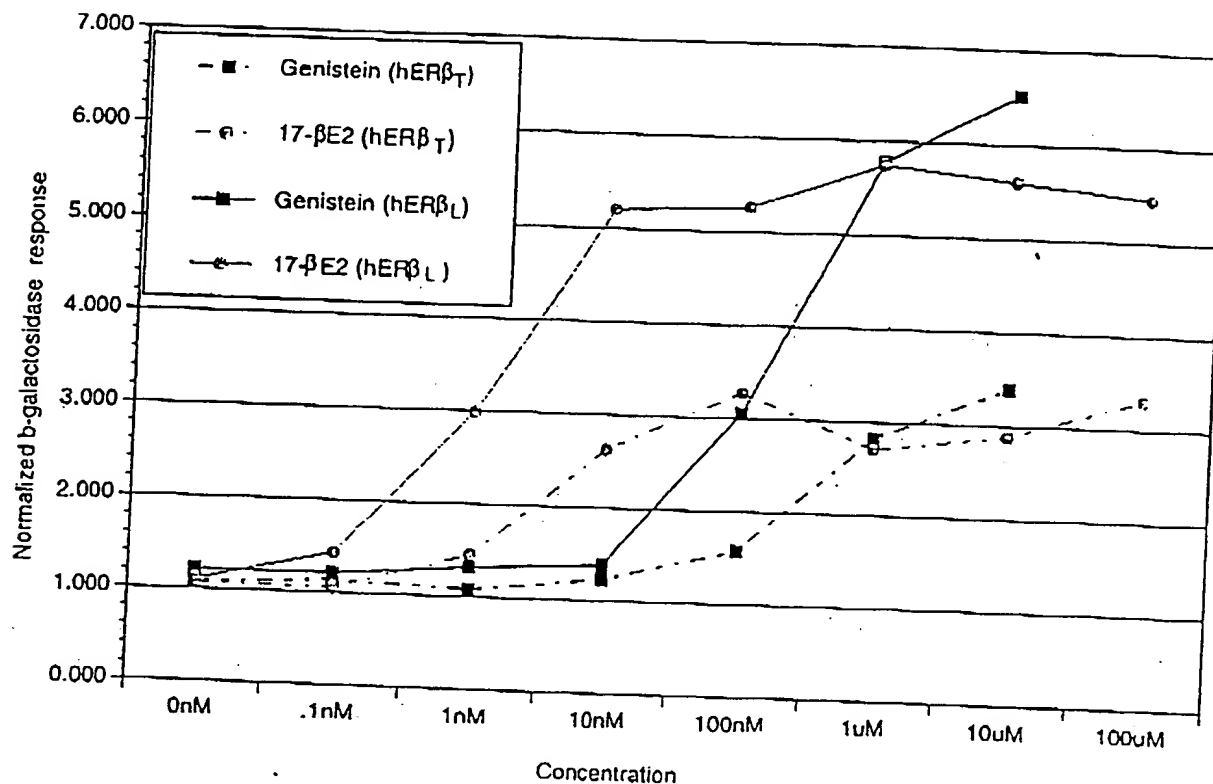
468



Transactivation of ERE reporter by ERβ_T and ERβ_L in HAECT-1 cells. Luciferase reporter constructs (20 μg) containing either the estrogen receptor DNA response element upstream of the TK basal promoter (ERE TK.LUC) or the TK basal promoter (TK.Luc) were transiently transfected into HAECT-1 cells (4x10⁶) with 5 μg of ER expression vector by electroporation. Cells were plated into 48 wells of a 96-well plate, rested for 4h, and treated overnight as indicated prior to luciferase determination. ERE TK.LUC values were normalized to TK.LUC values and are presented as mean ± S.E. (n=4).

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K269



Transcriptional activity of hERβ_T and hERβ_L in yeast. Yeast cells (BJ2168) were cotransformed with an ERE-LacZ reporter (YRpE2) and either a yeast vector (pYX242) expressing hERβ_T or hERβ_L. Transformed cells were grown in selective medium for 24 h at 30°C. Cells were treated with 17-β estradiol or genestein, at the indicated concentrations, for 3 h and then assayed for β-galactosidase activity.